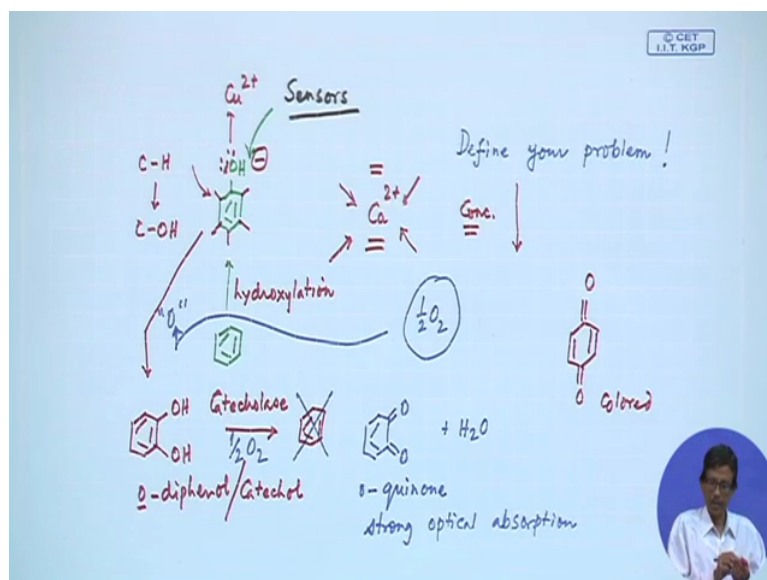


Course on Analytical Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology Kharagpur
Module 12
Lecture No 60
Applications (Contd.)

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Good morning everybody so in this last class of this particular course where we will be talking about the sensors will so finish first those about the sensors and where we are trying to handle or tackle uhh any sample whether you have a polymeric sample or a water sample or any environmental sample containing the phenol group, so to how to sense that but the basic idea behind all these things whenever we try to develop that sensing mechanism is that what are the molecules you are looking for because in this particular class also we will be talking something out of define your problem, how you so first thing that you have to define your problem.

How you define that particular problem and what are the methodologies you can follow so that you can identify that means initially detect and then quantify the total amount of the sample present in it. So right now we will finish this and then you just consider a very simple example because this is a very good source of metal ion and if you can track that how to track the calcium in diverse varieties of samples and what particular methodology you can follow and depending upon the very important thing the concentration range.

That is why if some methodology for your analytical chemistry is filled we should go for the next one and as we go down in all these concentration. So we should be very much careful starting from the sample getting the sample from the real world to finish the experiment and the data and the percentage and the porting.

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Many phenolic compounds occur in food products, especially those of plant origin, e.g. olive oil, wine, herbs and spices, coffee, various fruits and vegetables, and also milk and dairy products.

Phenol Oxidation by Water-Producing Oxidases and Oxygenases

Tyrosinase is a copper-containing monophenol monooxygenase

$$\text{Phenol} + \frac{1}{2} \text{O}_2 \xrightarrow{\text{Monooxygenase}} o\text{-diphenol} \quad (6.1)$$

$$o\text{-diphenol} + \frac{1}{2} \text{O}_2 \xrightarrow{\text{Catecholase}} o\text{-quinone} + \text{H}_2\text{O} \quad (6.2)$$

So what we were talking in our last class at the phenolic compounds and their presence in different species and we just basically go for the oxidised type of enzymes so it is oxidase so it is a tyrosinase because the name will also tell you that it is related to the tyrosinase which is an amino acid so if that amino acid tyrosine has the phenol unit itself, so inbuilt phenol unit is there in the tyrosine amino acid.

So copper will be required for this particular type of monophenol monooxygenase reaction and it has been identified the biological world that this phenolic can be oxidised by this copper so understanding everything from that biological world is also very important because once you know these things and if you critically see that okay that amino acid, it can be is obviously it is not a separate amino acid but it will be a part of the polypeptide chain or any other substrate containing the phenol groups.

So whenever you have the phenol groups that phenolic group can show some kind of activity and that basically give rise to the corresponding electron transfer reaction because if this is their so we know like your water molecules you have 2 lone pair of electrons on this phenol unit and when it is going for the (O⁻)(3:30) form that is the phenolic ion it can form a coordinate bond to a copper 2 centre. So if we go some electron transfer on this copper 2 plus

system and if we can go for some other type of reaction that means whether we can go for oxidation of this CH bond because all of them are CH you have 6 CH bond in the (C₆H₆) (3:54).

So likewise you have 6 instead of 6 you have 5, this is one, this is one, this is one so all 5 CH bond can be activated to convert this CH to COH. So it is a typical hydroxylation reaction so is a copper catalysed hydroxylation reaction on the phenol group and why we require this particular phenol group because the phenolic can approach this copper centre through this activity that means the weak interaction and ultimately the coordination bond which is forming between oxygen and copper can give rise to this sort of reaction.

So whenever you read it this sort of thing we should also think of why this particular reaction is going on, so these 2 reactions even if you consider that the first one is the phenol and when you have the monooxygenase you go for a diphenol reaction, so half of this O₂ molecule. So how much O₂ molecule is being consumed as we have seen earlier that can have some development of presence of O₂ by looking at the corresponding O₂ sensor that means the oxygen sensor.

So here if you can have some monitoring process uhh monitoring mechanism where we can see that this particular O₂ is consumed and if the concentration is going down is decaying so the amount of oxygen being consumed for this particular reaction, we can consider that particular reaction for its monooxygenase reaction are so is a monooxygenase reaction means that if you can have, even if you have this particular phenol group and if you go this one because this you can get from the (C₆H₆) (5:44). So the (C₆H₆) (5:46) if you have you can go for the corresponding hydroxylation reaction.

So is your hydroxylation reaction and this hydroxylation reaction is going to make you that CH to COH. So once you have this that means your phenol is there now this activated by the also because this can also be activated copper or any other metal ions, so if you have this so this phenol unit is there so where the second OH function, you can have you know that the options you have the ortho position, meta position and the para position but since it is OH uhh that copper activated process means copper should be nearby so only these 2 position that means ortho position will be activated and you get that diphenol so is therefore it is ortho diphenol what you will get.

So this is basically the corresponding oxygenase reaction and this basically this ortho diphenol is the another name of this is the Catechol. Why these are important because we

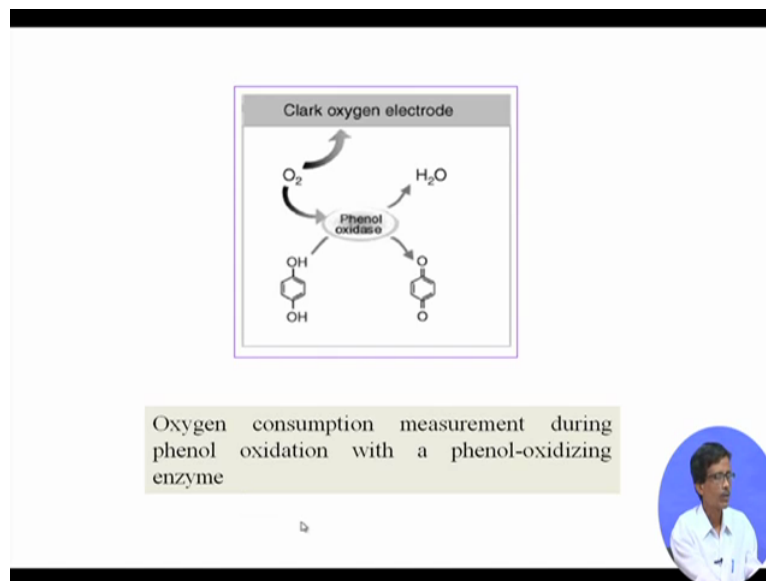
know that the several neurotransmitters are also able in our body and Catechol is also very good molecule for your potato peel also that it is browning when you know that potato peel is there if you remove the potato uhh covered skin, it again immediately goes for the corresponding browning, so the browning of the potato cover and the potato skin is due to the corresponding Catechol formation because it is highly intense in color when you go for this.

So something else unlike your this particular monooxygenase reaction since it is working on catechol, it would be called as the corresponding catecholase, so this catecholase will now work on it, so what will happen whether some other modification and all these things because this is basically the insertion of oxygen what we are getting from half O_2 , so this is basically going for this half O_2 , O_2 is being inserted over here CH will be COH. Now what will happen on this, so this will not go for any kind of the other third oxygen insertion on this, but it can go for its typical oxidation to its ortho benzoquinone, so is a ortho quinone or benzoquinone we call and is a strong optical absorption it can show.

So the darkening of the color and the strong optical absorption you can measure and you can find that there is a corresponding uhh reaction for this particular one, so this particular reaction take place so this H will go so again half of this O_2 can consume to give you the corresponding H_2O . So is basically these 2 reactions are one after another can take place so that the diphenol reaction formation of the diphenol as well as quinone formation this is simply the corresponding electron transfer reaction.

2 electron transfer of this catechol, catechol is going to first to semi-quinone and then semi-quinone to this ortho quinone. So if these 2 reactions are happening and when we have to monitor the corresponding presence of phenol or the corresponding presence of the diphenol in the medium so what we can do.

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If you have the phenol oxidase in your hand and that phenol oxidase can give rise to not only the biological thing what has been discovered earlier for your ortho diphenol unit that it can also go you give you the corresponding para benzoquinone formation because this para benzoquinone formation is also an interesting bio chemical reaction which is forming from the diphenol which is para diphenol and which is immediately forming and there also corresponding darkening in the color but the structure is wrong here, it is taken from some paper somewhere so this you have the double bond here and you have the double bond over there.

So this particular one like this you have a corresponding conjugation of the double bond here, here, here, so it is also coloured, so this colouring mechanism you have and then this thing that whenever you do this reaction whether it is your oxidise reaction or your catechol oxidase also the catecholase reaction. What is happening there, now you are consuming this O₂ because earlier we have seen that O₂ we can determine from Clark oxygen electrode.

If we have the Clark oxygen electrode, the O₂ concentration in this enclosed form or the enclosed area can be determined. Now in this O₂ is being consumed by the phenol oxidase and forming your water molecule so the reduction this O₂ concentration as well as the formation of this quinone, so quinone formation can monitor and reduction in this O₂ you can also monitor.

So these 2 monitoring process independently can give rise to the corresponding measurement that means oxygen consumption measurement using the phenol oxidation with the phenol

oxidising enzymes. So if we have the phenol oxidising enzymes, so consumption of O₂ you can monitor otherwise you can detect the corresponding concentration of the quinone formation in the reaction medium.

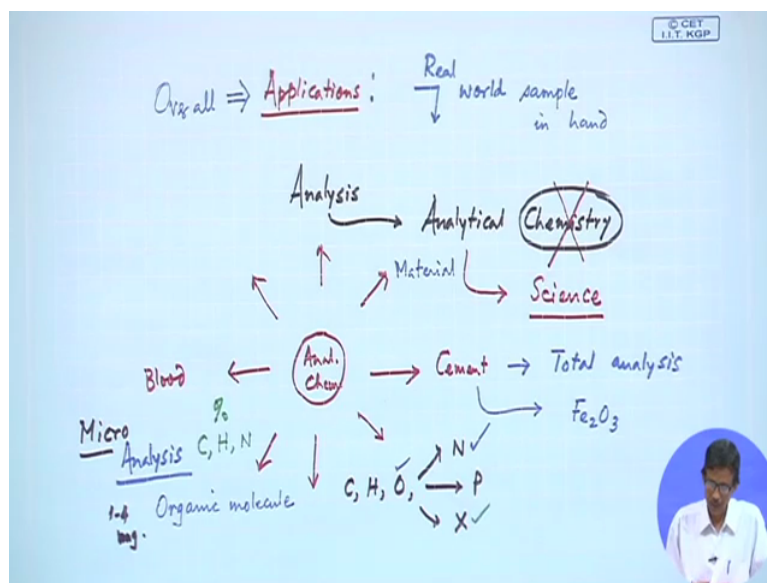
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APPLICATIONS OF PHENOLIC BIOSENSORS

(1) **Environmental control**, i.e. monitoring of the waste water treatment process and detection of contaminants in surface and ground water. Several tyrosinase, QH-GDH and CDH-based biosensors proved useful for assaying a phenol index in waste water, surface and river water during field tests.

(2) **Medicine and biotechnology**, i.e. determination of catecholamines in blood, brain and cell cultures, detection of cell numbers, and sensitive label detectors in enzyme immunoassays.

(3) **Food quality control**, i.e. quantification of phenols in olive oil, red wine or milk products.



So this basically give rise to the corresponding formation of these and we developed this likewise that of what we develop for your oxygen sensor or the glucose sensor, so definitely we should get some applications of these phenolic sensors. So now we slowly moved to in this particular last class basically, so initially we will just talk about the applications of the sensors and in broader respect to the electrochemistry and then how we handle this applications for some real-world sample.

If we have any real-world sample in hand, how we can monitor that particular one? So analysis of real sample which is also a practical aspect of your analytical chemistry, so for the phenolic biosensors, so every chapter we will have whenever you develop something whenever you know something, always you should think of the application because it is a typically applied signs.

So application is the first and foremost thing that is why we are one-time we are comparing uhh that who is your analytical chemist and chemical analysts, so analytical chemist will give the procedure of this measurement or the analysis and when the application is going on there is no need to develop that particular process at that particular point. Only how many samples you can handle, how many sample you can determine there are phenolic presence, so is very much required for environmental control.

So we will just talk about 3 such applications but carefully you listen this that these are very important in that how you can think of a subject where we are talking about so many theological things like that of your Faraday's current and the voltaic current and all these electron transfer and all these but ultimately how it is applicable to environmental control that means when in the countries where you have some strict environmental restrictions you cannot go beyond to that particular level.

So first thing will come is the monitoring of your wastewater you have to treat the waste water in only you can dump it, you can pass out that water in the drain line, so the whatever the water you are discarding even from our laboratories also for uhh laboratories like us so the Academy institution not the industrial institution or any R and D laboratory.

We use very small concentration of these materials for teaching purpose and little bit of our research purpose but we should not dump that water because we know that it is very much contaminated and if it goes and a proper care is not taken go through some open land or open drain, so the it goes down to your groundwater. So groundwater will also be contaminated if you are drain water is contaminated with phenol your groundwater at one point of time will definitely be contaminated by your phenol.

So let us see how we can detect that so for environmental control the phenolic biosensors will be very much useful for that purpose for the detection of the contaminates on surface as well as ground water, so as you see also the surface water, your lake water, you river water and the

groundwater, the groundwater is also being getting contaminated, so several tyrosinase you have then you a corresponding GDH.

So any dehydrogenase and CDH-based biosensors, so you have this do not bother about this term basically, the quinone base or the some other uhh have this reduced form of this based biosensors proved useful for assaying a phenol index in water it is a terminology very useful terminology that phenol is should be avoided in your water even for any kind of water, it may not be your drinking water so you have the phenol index in water on surface river water during field test, so you take the sample so is the biosensors that you have developed a very small one and if you can take this to the field.

So immediately in site determination of phenol is possible. So if it is like handheld PH meter, if it is some handheld PH meter, you can take that and the electrodes can be inserted on the water, whatever water is available over there in the field and you can tell us about the amount of phenol present in that particular water.

Then a broad application will be the area of pharmaceutical industry medicine and biotechnology because just now we are discussing about the catechol oxidase, so some molecules are also known as catecholamines and there are neurotransmitters for our neural activation for our neural signal in transfer. So you see that the name will immediately tell you, you know the amines like ammonia it is organic amines you know.

So catechol bound to some amine part so you have to have some part where you have the possibility that amine can be oxidised, the amine part can be oxidise in presence of your oxygen and catechol this can also be oxidise as we have just now we have seen it you need the enzyme catechol oxidase and if that enzyme is available and if you supply the required number of electrons on the electron it can give rise to the corresponding biosensors not only for your phenol, but also for your catechol and again for your catechol amines.

So when these are present or less diverse type of samples, so pathologist, the clinical uhh analytical chemist they also handle this blood samples, the brain samples and whatever samples can take out from the brain and also the cell cultures and for the detection of cell numbers and sensitive lab detection in the enzyme immunoassays, so enzyme immunoassays the enzymes is that is the catechol oxidase type of enzyme that can be (17:56) very nicely by knowing the responding information of your phenol biosensors.

Then large number of food material can have the corresponding species as your phenol species in it like your olive oil, red wine and milk product. Already I told you in our last class also, now through this biosensors if the concentration is very less say some microgram or nanogram level or the PPB or the PPM level parts per billion or parts per million level, so this can so be detected for assuring your food quality that no it has not been contaminated much with the level of phenol present in it with respect to your type that means whether you have your olive oil sample or a red oil sample or the milk products samples.

So whenever you read all these things all these information you can gather from any group or any research article, you should always have some idea how it is being detected, so what sort of analytical chemistry is involved over there and why you are only bothering to identify this because these are very important samples and very important real-life samples in our world.

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The slide is titled "Overall applications of Analytical Science" in an orange box. It contains three text boxes: a blue box with the text "Exact information about the composition and structure of matter. Art and science of determining what matter is and how much of it exists.", a green box with the text "It has applications including in forensic science, bioanalysis, clinical analysis, environmental analysis, and materials analysis.", and a red box with the text "Analytical chemists use their knowledge of chemistry, instrumentation, computers, and statistics to solve problems in almost all areas of chemistry and for all kinds of industries." A small number "4" is visible in the bottom right corner of the slide.

So now we just move on to the overall applications and if we just go for the overall applications is, so which is very important now that applications we are saying for electrochemistry of the bio electrochemistry development of the sensors. Now when you talk about this overall applications and we have to know that we go for analysis from the help of analytical chemistry.

So this analysis and analytical chemistry, so initially we will take a bit of knowledge and a knowledge is required the chemical knowledge or the chemistry knowledge is required but if we can apply this or any other areas all other areas so is basically it can be considered as a science, it should not be restricted within chemistry because we start with chemistry but when

you are ending up with something it is covering all the different areas, so any analytical chemistry we open now you will find there are large number of areas it can cover and which it can go or its applications.

So is a centrally you can have the corresponding analytical chemistry but when you apply to get some analysis, suppose if we have a cement sample in one hand and another and you have the blood sample so how you correlate this things, how your knowledge of analytical science can be helpful to identify one of the component that means you are not analysing that means some of this we consider as the total analysis and sometimes we need to go for only the presence of Fe_2O_3 the amount of iron because iron is not wanted to be present in good quality of cement for building materials as a good building materials.

So if you see that even if you do not know the area where you are because the cement you know the chemists are handling, the civil engineers are handling, the architects are handling and people who are making this also in the industry they also handling cement, so they should know the purity of this material and whether you have uhh the permissible amount of Fe_2O_3 in it and sometimes we can go for the total analysis for all these things.

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The slide is titled "Overall applications of Analytical Science" in a red box. It contains three text boxes: a blue box defining analytical science as the art and science of determining matter's composition and structure; a green box listing applications in forensic science, bioanalysis, clinical analysis, environmental analysis, and materials analysis; and a red box stating that analytical chemists use chemistry, instrumentation, computers, and statistics to solve problems in all areas of chemistry and industries. A small number '4' is in the bottom right corner.

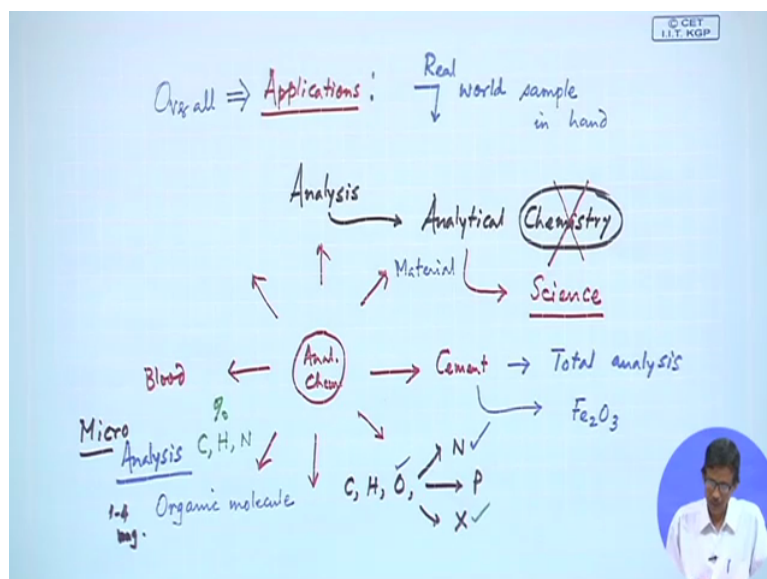
Overall applications of Analytical Science

Exact information about the composition and structure of matter. **Art and science** of determining what matter is and how much of it exists.

It has applications including in forensic science, bioanalysis, clinical analysis, environmental analysis, and materials analysis.

Analytical chemists use their knowledge of chemistry, instrumentation, computers, and statistics to solve problems in almost all areas of chemistry and for all kinds of industries.

4



So if you go for this overall application of this what we get, we get the exact information about the composition and structure of the matter, so initially you will get the composition means the percentages we require to know so we get what is that go for the analysis this analysis again we are talking about the analysis and suppose we have a small organic molecule we have prepared in the laboratory.

So what we can go for the first analytical chemistry you can apply to know this analytical molecule, that molecule organic molecules, so we apply different spectroscopic techniques little bit understanding about its structure and its bonding and all these things but the very basic thing is we can do cause these are very small amount only, few milligrams will be available.

So 1 to 4 milligram if they are available for analysis milligram, if they are available for analysis so what you get is that we can go for a micro analysis and earlier we are knowing only that if you are organic molecule we all know that the organic molecule is forming with carbon, hydrogen, oxygen you can have also nitrogen, you can have also phosphorus and you can have halogens.

So these all are there so in the laboratory also the amount is more you know from your school days that you can analyse this corresponding organic molecule for its presence of nitrogen and presence of halogen also be the first thing we know learn it from our school days at in organic molecules how to detect the nitrogen and the halogen but if you go for the micro analysis and which is automated one so it gives rise to the percentages of initially we call it also the CHN analyser.

So Perkin Elmer or any other instrument making company they are making us giving us the CHN analyser, so CHN analyser immediately we go for this particular analysis it can tell you the percentage of carbon, percentage of hydrogen and percentage of nitrogen again taking sample in the range of 1 to 4 milligram which is not possible detect conventionally with a sample of this range for the percentage analysis of all 3 together, so micro analysis is very important with regard to this particular one.

So basic thing what we can use is the analysis of any molecule containing carbon, hydrogen, oxygen, nitrogen and all this so you go from here that means if you instead of this cement sample any material you have also and once we know that the materials has oxygen you know so you can detect that also a particular presence of oxygen at particular material.

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The slide is titled "Overall applications of Analytical Science" in a red box. Below the title are three text boxes: a blue box with text about exact information and art/science, a green box with text about applications in forensic science, bioanalysis, clinical analysis, environmental analysis, and materials analysis, and a red box with text about analytical chemists using chemistry, instrumentation, computers, and statistics. A small number '4' is in the bottom right corner.

Overall applications of Analytical Science

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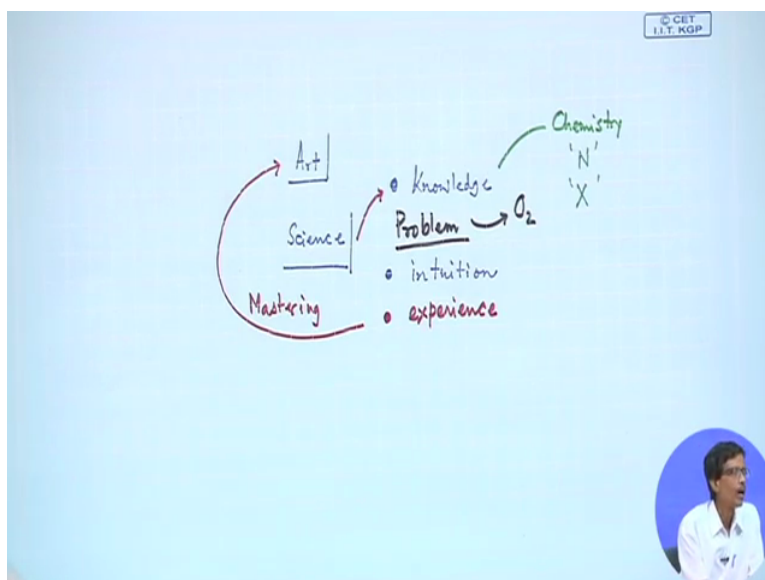
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So the composition and structure because the spectroscopic techniques will tell you structure if you have double bond how many double bonds are there, if you have ethylene sample in hand, if you have the polythene sample in hand and if you have also the living world sample like beta carotene in your hand. So how you can detect all this things in terms of the corresponding values in the Lambda Max values and ν_{max} values.

So this is together both these are together at means it is art that means it should be mastered enough. So it is an art and science so what scientific knowledge you must have the this analysis is that a very little amount of chemical knowledge or the chemistry knowledge you must have a basically it is a typical art that how you develop this thing that means with your

knowledge that means some amount of knowledge you must have, so if we just side-by-side if we see philosophically what is the philosophy behind all this things?

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Is that that if you have this particular one in terms of your while we are talking it as art and science, so if you have this particular one that means you must require some knowledge and knowledge what we know that this particular knowledge what you can gather from chemistry because it starts with the chemistry finding out of whether you have nitrogen in any sample or halogen in organic sample.

So this chemical knowledge is there then the problem will come that you have to define the problem, how you define the problem? That means whether you are trying to determining development of the sensors like the developing of sensors for O₂ determination, so you define the problem and you try to solve it in a way that how you can get this particular information related to this O₂ concentration than that means your intuition should be applied but how you tackle this thing, how you apply your knowledge to get this thing and once you do it is not that only one sample you analyse and you get good results.

Even if you handle instruments that means it can be simple instrumental method of analysis, so you must be experienced enough, so your experience is important, so scientific paths related to your knowledge and your this experience and mastering of this thing will be related to our art of this particular subject. So you have to be mastering yourself for do all these things, so that is why we can consider it as a art and science o determining what matter is and how much it is exist in that particular sample.

And also it has applications including in forensic science, bioanalysis, clinical analysis, environmental analysis and materials analysis and as I told and I repeatedly say every day almost that what are the areas you can handle. So use their knowledge of chemistry that is why we are using your knowledge of chemistry first start with that particular knowledge then the instrumentation, if we consider that okay I cannot handle by simple burette pipette analysis or titration of the gravimetric estimation, you have to go for the instrumentation of what instrumentation you should need whether you require a visible spectrophotometer or a gas chromatography or a mass spectrometer.

Then some small amount of knowledge you required for handling those computers because most of these instruments are now software driven so how to run those software and how to run the protocol for this because the computer will acquire these things like your cycling voltammetry is also your computer, you will see the screen but how to store the data, so not only the image what you are storing but also the data you have to store it and the statistics because analysis you have to do on those data that means when you go for the corresponding use of simulation packages side told you earlier in one time that simulation packages are there and it should be with you such that you can extract out some good information from that particular spectrum or particular voltammogram.

When you handle the voltammogram know all the data including your kinetic parameters you can extract out from the lines shape of the voltammogram. To require the statistics to solve the problem in almost all the areas of mystery forget about chemistry so in all areas of science also several scientific areas you can handle even you can go for some analysis of the art object, analysis of historical object has been found from the Earth and all kind of industries can be served with this particular information and the knowledge.

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The slide features three text boxes with the following content:

- Top box (orange background): "Their measurements are used to assure the safety and quality of food, pharmaceuticals, and water"
- Middle box (green background): "To assure compliance with environmental and other regulations. To support the legal process."
- Bottom box (blue border): "To help physicians to diagnose diseases."

In the bottom right corner, there is a circular portrait of a man with glasses, wearing a white shirt, against a blue background.

So their measurements are used to assure also the safety and quality of food, pharmaceutical and water, so analytical chemist also has some role played to the society that you assure that the food quality is maintained, whenever you take some drinking water always you should know this is basically awareness, some basic and fundamental knowledge and information that what amount of potassium and sodium ions you are taking with that particular drinking water and you are not consuming by mistake any arsenate or (AsO_4^{3-}) ion from your water.

Similarly the pharmaceutical uhh material also you always know that whenever you have a material whatever you talking about is not 100 percent pure, so some percentage of impurity is there and what sort of impurity? Even it is a dye for making the tablet or the capsule or other extraneous matter what is present over it you should know that and then the quality of the water always we handle water for making food preparing all this pharmaceutical products and food also.

So you have the corresponding restriction and the compliance with the environmental corresponding uhh restriction what is given the environmental protection agencies and other agencies, the regulatory authorities, so with environmental and other regulations, so we must have this regulation that your drinking water should not contain more than 3 ppm or 4 ppm potassium as well as sodium so you should follow that whenever you buy it particularly not that what are available from your river or groundwater but when you buy it from some companies water who are making for our drinking water or the bottled water.

So they should follow this environmental restriction and it should they should print on what was basically whatever samples you can have and to support the legal process if something is happening, as the forensic science, if somebody is coming to you and need some help whether something is contaminated, arsenic something is contaminated with lead or something is contaminated with cadmium.

How you help it? So the legal process can also be help so the legal advisers, the lawyers, the judges all should have some very basic understanding about the analytical science because otherwise you cannot know at what amount of cadmium you can have, what amount of lead you can have your sample whether it is a medicine, whether is a food material, whether it is what sample.

So to help the physician also so it is a direct application that you can have those positions diagnosed sometimes the diseases. You know that the percentage of your glucose in the blood then the amount of your sodium or the potassium or the calcium these are the very simple and the very basic things what you can help people to know that you can determine from any such samples.

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Common clinical chemistry tests include:

Electrolytes

Chloride is an essential electrolyte, trafficking in and out of cells through chloride channels and playing a key role in maintaining cell homeostasis and transmitting action potentials in neurons.

Silver chloride electrodes are used by many applications of biological electrode systems such as biomonitoring sensors as part of electrocardiography (ECG) and electroencephalography (EEG)

The slide features three text boxes: a yellow one at the top, a light blue one in the middle, and a light green one at the bottom. A small circular inset in the bottom right corner shows a man in a white shirt speaking.

So what we get that electrolyte just now we are talking about that electrolytes are your sodium chloride or potassium chloride simply we can have, so when you go to any pathological center, the common chemical uhh clinical chemistry they do they take go for the test to analyse the uhh corresponding electrolyte presence. So one such electrolyte is your chloride because always we talk about the corresponding concentration of sodium and

potassium but we neglect the corresponding anion of this because all these things we take as the corresponding sodium salt.

So chloride will automatically come if you talk in terms of the sodium and potassium, the chloride ion the Cl minus, so this can also be considered as a good electrolyte trafficking in and out of cells so we are talking in terms of the corresponding sodium potassium pump and all these things but we do not talk much about the corresponding chloride concentration because you have also the chloride channels like sodium potassium and calcium channels and the cells homeostasis and transmitting action potentials to the neurons due to the presence of varying chloride concentration like of your corresponding proton concentration and the proton motive force.

And when you talk in terms of this different electrodes as silver chloride electrodes are used in many applications we know that the silver silver chloride electrodes so this chloride part is coming over here so we get the silver chloride electrode is a half-cell electrode we know and such as biomonitoring sensors also not that directly you are determining corresponding O₂ concentration or the glucose concentration or the phenol concentration but it can be able to sense something as part of the things from our childhood basically you are been listening about the electrocardiography and electroencephalography the ECG and EEG always we know.

So these 2 things are since we are talking about electro and cardiogram is the cartographic we do, so is basically the electron transfer means you must have some electrodes system so 12 electrodes basically we use for measuring ECG, so whenever we have some idea about electro or you can also think of sort of electrode or is using for measuring your ECGs, okay. Thank you very much.